

REVIEW ARTICLE

Control of Programmed Cell Death in Normal and Leukemic Cells: New Implications for Therapy

By Leo Sachs and Joseph Lotem

NORMAL DEVELOPMENT requires the regulation of cell viability, growth, and differentiation to different lineages. Cell death occurs at specific times and sites in normal embryonic development, hence the name programmed cell death, which has also been called apoptosis.^{1,2} This form of cell death by committing suicide is characterized by DNA degradation into fragments with sizes of one or more nucleosomes (DNA ladder) and by specific morphologic changes including chromatin condensation and nuclear fragmentation.^{1,2} Programmed cell death occurs not only during normal embryonic development but also in normal adults. Can this program be suppressed so as to decrease the loss of normal cells in the body and what happens when normal cells are changed into cancer cells? Are cancer cells immortal or do they maintain the program for cell death? If they maintain this program, how can it be activated so that cancer cells will commit suicide? The present article will mainly describe results on the control of programmed cell death in normal and leukemic hematopoietic cells that have been used as a model system to answer these questions.

CELL VIABILITY AND CELL GROWTH CAN BE DISSOCIATED

Different types of cells in the body, and probably all cell types, require specific factors to remain viable (viability factors)³⁻¹⁸ and removal of such factors results in programmed cell death (apoptosis) (Fig 1). It has been shown with myeloid hematopoietic cells that these viability factors, which suppress apoptosis, are required throughout the differentiation process from immature cells^{3,13,16} to mature granulocytes,¹² eosinophils,^{12,16} and macrophages.^{10,11} There are viability factors such as the four hematopoietic colony-stimulating factors (CSFs), including interleukin-3 (IL-3), which also function as growth factors. However, IL-6 and IL-1 can induce viability in normal immature myeloid cells without inducing growth.¹³ The tumor promoting phorbol ester 12-O-tetra-decanoyl-phorbol-13-acetate (TPA) can also induce viability by suppressing apoptosis in these cells without inducing growth.¹⁹ The *bcl-2* gene can suppress apoptosis without inducing growth in viability factor-dependent cells after removal of the viability factor.^{20,21} The results show that although there are viability factors that can also induce growth, viability and growth are separately regulated processes. The hematopoietic factors that induce viability and growth²⁴⁻³⁰ are part of a network of regulatory cytokines.^{28,32} It will be interesting to determine whether the viability factors that also function as growth factors induce growth directly, or do so indirectly by switching on production of other factors that induce growth.

SUPPRESSION OF PROGRAMMED CELL DEATH IN LEUKEMIC CELLS BY VIABILITY FACTORS

There is no evidence that cancer cells are immortal. Tumor cell lines some of which have been subcultured for

years contain cells that die by apoptosis. These lines can be subcultured for long periods not because the tumor cells are immortal, but because more cells survive for some time and multiply than die. As long as this balance is maintained in favor of the multiplying cells, a tumor cell line can be subcultured. Cancer cells still maintain the suicide program for cell death that can be activated by different agents and suppressed by viability factors. This has been clearly shown in experiments with myeloid leukemia. Certain myeloid leukemic cells are growth-factor independent and do not require a hematopoietic cytokine for cell viability and growth. However, some of these leukemias can still be induced to undergo terminal-cell differentiation by cytokines such as IL-6, IL-3, or granulocyte and macrophage CSF (GM-CSF) resulting in suppression of the leukemic state.^{9,28,32} Even before terminal differentiation these leukemic cells regain the normal requirement for hematopoietic cytokines to maintain viability.^{4,7,19} Withdrawal of these cytokines leads to cell death by apoptosis in the differentiating leukemic cells.¹⁹ Rescue from apoptosis in the differentiating leukemic and in normal myeloid cells by these hematopoietic cytokines or by the tumor promoting phorbol ester TPA was suppressed by amiloride analogs that inhibit the Na⁺/H⁺ antiporter.¹⁹ The use of these analogs has indicated that Na⁺/H⁺ exchange is involved in the suppression of programmed cell death in normal and leukemic myeloid cells by viability factors.^{19,33}

Induction of programmed cell death in cancer cells can also be achieved without inducing a differentiation-associated viability-factor-dependent state. A variety of agents including cytotoxic cancer chemotherapy compounds,^{1,2,34-36} transforming growth-factor- β 1 (TGF- β 1),^{34,37} heat shock,³⁸ antibody to certain cell-surface antigens,^{38,42} and transfection with the tumor-suppressor gene wild-type p53^{43,44} can induce apoptosis in cancer cells. Normal hematopoietic cytokines such as GM-CSF (Table 1) granulocyte-CSF (G-CSF), IL-3, and IL-6 suppressed this induction of apoptosis by cytotoxic cancer therapeutic agents or TGF- β 1,³⁴ and IL-6 suppressed apoptosis induced by wild-type p53.⁴³ IL-6 also reduced the susceptibility of

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Submitted January 11, 1993; accepted March 24, 1993.

Supported by the Catherine Lewis Foundation, the National Foundation for Cancer Research (Bethesda, MD), the Hermann de Stern Foundation, the Jerome A. and Estelle R. Newman Assistance Fund, and the Ebner Foundation for Leukemia Research.

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0006-4971/93/8201-0039\$3.00/0

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0006-4971/93/8201-0039\$3.00/0

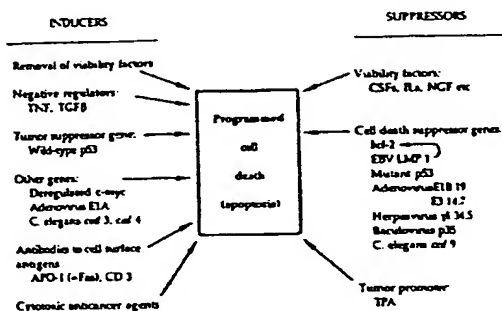


Fig 1. Inducers and suppressors of programmed cell death. References in the text.

Fanconi anemia cells to the cytotoxic action of mitomycin C⁴³ and IL-4 suppressed induction of apoptosis by hydrocortisone in B-cell chronic lymphocytic leukemia cells (B-CLL).⁴⁶ Even in leukemic cells that are not factor dependent for viability, the viability-inducing hematopoietic cytokines can suppress induction of apoptosis by different agents.³⁴

The activation of apoptotic cell death when there are insufficient amounts of viability factors has important implications not only for the regulation of cell viability during normal development, but also for the development and metastasis of malignant cells and the ability to eliminate cancer cells by different forms of therapy. A change from viability and growth-factor independence to a viability-factor-dependent state may be used to eliminate cancer cells by apoptosis if the availability of the required viability factor(s) is reduced. Injection of antibody to nerve growth factor (NGF), which is a viability factor for certain cells of the nervous system, resulted in *in vivo* destruction of the neurons that are NGF-dependent.³ It has also been found in multiple myeloma where the cells are IL-6 dependent for viability^{47,48} and the severity of the disease is correlated with the amount of IL-6 in the circulation,⁴⁹ that the use of monoclonal anti-IL-6 or anti-IL-6 receptor antibody reduced tumor-cell development *in vitro* and *in vivo*.^{49,50} In human myeloid leukemias, most cells taken from patients are factor dependent for viability *in vitro*.³¹ It will be interesting to determine whether a similar strategy of reducing the level of viability factors will be successful in the therapy of leukemia and other types of cancer by inducing the suicide program in the cancer cells.

REGULATION OF PROGRAMMED CELL DEATH BY THE GENES P53, C-MYC, AND BCL-2

There are genes that induce or suppress programmed cell death. Wild-type p53,^{43,44} adenovirus E1A,^{32,33} and *ced-3* and *ced-4* in the nematode *Caenorhabditis elegans*,^{34,35} can induce apoptosis. Deregulated *c-myc* can enhance apoptosis under certain conditions.^{36,37} Genes for cell-surface antigens such as APO-1 (=Fas) and CD 3³⁹⁻⁴² mediate apoptosis when cells expressing these antigens are incubated with the appropriate antibody. Other genes including *bcl-2*,^{20-23,38}

mutant p53,³⁴ adenovirus E1B 19Kd^{32,33} and E3 14.7Kd,³⁹ herpes-simplex virus 1 γ , 34.5,⁴⁰ baculovirus p35,⁴¹ and *C. elegans ced-9*^{34,35} can suppress apoptosis (Fig 1). We will mainly discuss the control of apoptosis by p53, *c-myc* and *bcl-2* as a model system to study genes that regulate programmed cell death in normal and malignant mammalian cells.

Deregulated expression of the tumor-suppressor gene wild-type p53 can induce apoptosis in myeloid leukemia and other cancer cells.^{43,44} This showed that a tumor-suppressor gene can suppress malignancy by inducing apoptosis and this may also apply to some other tumor-suppressor genes. It has been shown that DNA-damaging agents induced an increase in wild-type p53 expression⁴² and that apoptosis in normal epithelial cells in the prostate after male hormone depletion and in mammary-gland involution was also associated with increased expression of wild-type p53.^{43,44} Myeloid precursors from mice with no wild-type p53 are more resistant to induction of apoptosis at a low concentration of viability factors and after irradiation or heat shock (J.L., L.S., to be published). These results show that wild-type p53 is involved in the normal process of apoptosis presumably by its ability to act as a transactivator^{45,46} or inhibitor⁴⁶⁻⁴⁹ of transcription of other genes. Viability factors may function by decreasing expression of wild-type p53. However, there must also be alternative pathways to suicide by programmed cell death, because apoptosis can be induced in cells containing no wild-type p53 such as leukemic HL-60⁵⁰ and M1^{34,43} cells, and thymocytes from mice with no wild-type p53 are more resistant to induction of apoptosis by irradiation but not to induction of apoptosis by dexamethasone (J.L., L.S., to be published).

Apoptosis under growth-restrictive conditions in certain nonmalignant factor-dependent myeloid cells and Rat-1 fibroblasts can be enhanced by deregulated expression of *c-myc*.^{36,37} In leukemic cells, deregulated expressions of *c-myc* did not induce apoptosis but enhanced cell susceptibility to various apoptosis-inducing treatments³⁸ (Table 2). Deregulated expression of mutant p53 neither induced apoptosis⁴³ nor changed the susceptibility of leukemic cells to apoptosis-inducing treatments³⁸ (Table 2). However, deregulated

Table 1. Suppression by GM-CSF of Induction of Programmed Cell Death in 7-M12 Myeloid Leukemic Cells by Irradiation and Cancer Chemotherapy Compounds

| Therapeutic Agent | Dose | % Apoptotic Cells* | |
|-----------------------|------------------|--------------------|----------|
| | | - GM-CSF | + GM-CSF |
| γ -irradiation | 400 R (65 R/min) | 40 | 3 |
| Adriamycin | 0.5 μ g/mL | 58 | 6 |
| Methotrexate | 100 μ g/mL | 45 | 18 |
| Cytosine arabinoside | 5 μ g/mL | 28 | 2 |
| Vincristine | 0.1 μ g/mL | 15 | 2 |

* The percent apoptotic cells was determined 5 hours after adding the therapeutic agent. GM-CSF was added at 5 ng/mL. GM-CSF also suppressed induction of apoptosis in these leukemic cells by some other compounds including TGF- β 1, cycloheximide, and sodium azide.³⁴

Table 2. Control of Programmed Cell Death by Deregulated Oncogenes and a Tumor-Suppressor Gene

| Deregulated Expression of | Apoptosis | | |
|---------------------------|-----------|-------------|-------------|
| | Induction | Enhancement | Suppression |
| Wild-type p53 | + | - | - |
| c-myc | - | + | - |
| Mutant p53 | - | - | + |
| bcl-2 | - | - | + |

* Mutant p53 suppresses the enhancement of apoptosis by deregulated c-myc.³⁹ Other references in the text.

mutant p53 suppressed the apoptosis-enhancing effect of deregulated c-myc³⁸ (Table 2). Thus, an oncogene such as mutant p53 can promote the viability of cells expressing deregulated c-myc after treatment with apoptosis-inducing agents. In addition to enhancing apoptosis, deregulated c-myc can also induce cell proliferation³¹ and inhibit differentiation.³⁴ Thus, the suppression of the apoptosis-enhancing effect of c-myc by mutant p53 can allow expression of these other functions of c-myc. bcl-2 also suppresses the apoptosis-enhancing effects of deregulated c-myc.^{71,72} Therefore, c-myc and mutant p53 can cooperate in tumor development as occurs with c-myc and bcl-2.⁷³ This possibility is supported by the finding that noncancerous skin fibroblasts from cancer-prone patients with the Li-Fraumeni syndrome carrying a germ-line mutation in p53^{74,75} show overexpression of c-myc.⁷⁶ It can be suggested that the poor prognosis of gastric, colorectal, and lung carcinoma patients that express high levels of mutant p53⁷⁷⁻⁷⁹ may be associated with the ability of mutant p53 to inhibit apoptosis, as well as with the elimination of wild-type p53 that can induce apoptosis^{43,44} or growth arrest.^{45,46} Cell transformation by v-abl or Bcr-abl also seems to require overexpression of c-myc⁸⁰ and it will be interesting to determine whether abl and other oncogenes can inhibit the apoptosis enhancing effect of deregulated c-myc.

The ability of bcl-2 to suppress apoptosis^{20,23,34} also influences the susceptibility of cancer cells to apoptosis-inducing therapy. A study of different leukemias has shown that a high level of expression of regulated bcl-2 was associated with a high resistance to various apoptosis-inducing cancer chemotherapy compounds (Table 3).³⁴ There was no association between the susceptibility of these leukemias to induction of apoptosis and the level of regulated c-myc expression or cell competence for differentiation.³⁴ The difference in susceptibility to chemotherapy compounds in leukemias with differences in expression of bcl-2 was not caused by differences in the activity of multidrug resistance (MDR) genes³⁴ that code for the P-glycoprotein (P-GP) transporter that regulates drug accumulation inside the cells.^{81,82} Thus, screening for expression of bcl-2 may be useful to characterize leukemias and other types of cancer cells regarding susceptibility to induction of apoptosis by therapeutic agents.³⁴

Induction of differentiation in leukemic cells which is associated with development of a viability-factor-dependent state,^{4,7,19} was associated with a decrease in bcl-2 expression.³⁴ This differentiation-associated decrease in expression of bcl-2 appears to be a property of the normal myelo-

monocytic and B-lymphocytic lineages.⁸³⁻⁸⁵ In addition, expression of high levels of bcl-2 in germinal center cells of lymphoid follicles and in the thymus was topographically restricted to areas in which the cells are long-lived and display low susceptibility to apoptosis.⁸³ Suppression of apoptosis in normal germinal center cells expressing low bcl-2 by antibody to the surface antigen CD 40 or by a 25-Kd fragment of CD 23 protein plus IL-1 was associated with induction of bcl-2 expression⁸⁴ and induction of bcl-2 also occurred in B-CLL leukemic cells protected from apoptosis by IL-4.⁸⁶ Therefore, it appears likely that bcl-2 downregulation during normal differentiation is involved in determining the limited life span of mature granulocytes and antibody-producing plasma cells. The results also suggest that suppression of apoptosis in normal cells by viability factors is associated both with downregulation of wild-type p53 and upregulation of bcl-2 expression. Viral genes such as the Epstein-Barr virus (EBV) LMP-1 can induce expression of bcl-2 in infected cells and thus protect the cells from apoptosis⁸⁷ (Fig 1). It will be interesting to determine whether the other viral genes that confer resistance to apoptosis^{32,33,39,60} (Fig 1) are also mediated by expression of bcl-2. It remains to be determined which other cellular genes are involved in protection against apoptosis in mammalian cells, and to search for ways to downregulate the expression or activity of bcl-2 and mutant p53 so as to increase the susceptibility of cancer cells to therapy.

Protection from apoptosis by viability factors,^{3,20,33,34,43} tumor promoters such as TPA^{19,22,80} that protect cells by a different pathway than G-CSF, M-CSF, IL-3, or IL-6,^{19,84} a gene such as bcl-2,^{20,23,34} oncogenes such as mutant p53,³⁸ and certain viral genes,^{32,33,39,60} or by elimination of genes such as wild-type p53 that induce apoptosis^{43,44} (Fig 1), can therefore be involved in the pathogenesis of cancer. Inhibition or delay of cell death together with blocks in cell differentiation in cells affected by a carcinogenic agent, will obviously enhance the probability of further changes such as constitutive activation of growth-promoting oncogenes that will eventually lead to the malignant phenotype.

VIABILITY FACTORS AND THERAPY

The use of hematopoietic cytokines that also function as viability factors has already proved to be of clinical value.

Table 3. Expression of bcl-2 and Induction of Programmed Cell Death by Cancer Chemotherapy Compounds in Different Leukemias

| Leukemias | Expression of bcl-2 | Induction of Apoptosis |
|-----------|---------------------|------------------------|
| M1 | ++ | ± |
| 7-M12 | ± | ++ |
| 1 | ± | ++ |
| 6 | ± | ++ |
| 10 | ± | ++ |

M1, 7-M12, 1, 6, and 10 are five independently derived myeloid leukemias. The association between expression of bcl-2 and susceptibility to apoptosis was found with induction of apoptosis by the cancer chemotherapy compounds adriamycin, cytosine arabinoside, and methotrexate. This association was also found with induction of apoptosis by cycloheximide or heat shock.³⁴

Treatment with a cytokine such as G-CSF is of clinical value in treating myelosuppression after cytotoxic cancer therapy and other procedures that cause immune depression, and in improving engraftment and recovery after bone marrow (BM) transplants.^{28,30,31} Normal granulocyte development can be induced in cells from patients with congenital agranulocytosis by a CSF^{32,33} and treatment with G-CSF has proved useful in the therapy of this genetic disease.³⁴ G-CSF induces cell viability and cell growth. The therapeutic effect of G-CSF may include the increased survival of immature and mature granulocytes. The use of other cytokines that act as viability factors may be of therapeutic value by suppressing apoptosis and thus increasing postinjury survival of other cell types including normal neurons.^{3,13,35} Treatment with viability inducing cytokines may also be useful to suppress the lethal effect of irradiation and other cytotoxic agents on normal cells, and to suppress the lethal effect of infection of normal cells with viruses including those that cause AIDS.

Because viability factors can suppress apoptosis in cancer cells, the preferential development and metastasis of leukemia and other types of cancer cells in specific tissues in the body may be caused by better protection against apoptosis at these sites because of the presence of appropriate viability factors. This protective effect of viability factors can also explain the poorer complete remission rates after chemotherapy³¹ in human acute myeloid leukemia (AML) patients whose cells have a higher responsiveness to hematopoietic viability factors *in vitro*. In view of the ability of viability factors to inhibit apoptosis induced by a variety of chemotherapeutic agents,^{34,36} endogenous viability factors may decrease the effectiveness of cytotoxic therapy. Thus, the clinical use of cytokines to correct chemotherapy- or radiotherapy-associated myelosuppression should be carefully timed to avoid protection of cancer cells from the apoptosis-inducing action of therapeutic compounds.³⁴ This could avoid cases where the prognosis of leukemia patients receiving GM-CSF before or during chemotherapy is worse than in patients treated with chemotherapy alone.³⁶ Therefore, it can be suggested that reducing the level or activity of viability factors may be clinically useful. This reduction could induce the suicide program in factor-dependent leukemic cells and reduce the protective effect of viability factors in leukemic cells treated with cytotoxic therapy. One way of reducing the level or activity of viability factors is by using antibody to these factors or to their receptors. In view of the ability of different hematopoietic cytokines to fulfill similar functions and the network of interactions between these cytokines,^{28,32} reducing their availability may require the use of more than one antibody. Another way of reducing the level of viability factors would be to use agents that suppress the synthesis of these factors such as hydrocortisone.³⁷ Such a suppression may explain how high doses of methylprednisolone administered during induction of remission and maintenance therapy increased complete remission rates and prolonged their duration in children with AML.³⁸ It will be interesting to see further results of clinical trials with a reduction of viability factors. The suggestion to combine chemotherapy with reduction of

viability factors is different from the suggested use of viability and growth-inducing cytokines to increase the frequency of cycling leukemic cells and thus make the leukemic cells more susceptible to the cytotoxic action of cycle-specific chemotherapeutic agents.^{99,100} Increase in the level of cytokines such as IL-6,¹⁰¹⁻¹⁰⁴ IL-3, or GM-CSF¹⁰⁵ can be useful in some cases by directly^{101,102,105} or indirectly¹⁰³⁻¹⁰⁵ suppressing tumor development. Thus, different circumstances may require the reduction or increase of certain cytokines to devise a more effective therapy.

SUMMARY

Programmed cell death (apoptosis) is a normal process by which cells are eliminated during normal embryonic development and in adult life. Disruption of this normal process resulting in illegitimate cell survival can cause developmental abnormalities and facilitate cancer development. Normal cells require certain viability factors and undergo programmed cell death when these factors are withdrawn. The viability factors are required throughout the differentiation process from immature to mature cells. Although many viability factors are also growth factors, viability and growth are separately regulated. Viability factors can have clinical value in decreasing the loss of normal cells including the loss that occurs after irradiation, exposure to other cytotoxic agents or virus infection including AIDS. There is no evidence that cancer cells are immortal. Programmed cell death can be induced in leukemic cells by removal of viability factors, by cytotoxic therapeutic agents, or by the tumor-suppressor gene wild-type p53. All these forms of induction of programmed cell death in leukemic cells can be suppressed by the same viability factors that suppress programmed cell death in normal cells. A tumor-promoting phorbol ester can also suppress this death program. The induction of programmed cell death can be enhanced by deregulated expression of the gene *c-myc* and suppressed by the gene *bcl-2*. Mutant p53 and *bcl-2* suppress the enhancing effect on cell death of deregulated *c-myc*, and thus allow induction of cell proliferation and inhibition of differentiation which are other functions of deregulated *c-myc*. The suppression of cell death by mutant p53 and *bcl-2* increases the probability of developing cancer. The suppression of programmed cell death in cancer cells by viability factors suggests that decreasing the level of these factors may increase the effectiveness of cytotoxic cancer therapy. Treatments that downregulate the expression or activity of mutant p53 and *bcl-2* in cancer cells should also be useful for therapy.

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